Arterial Tissue Mimics for Studying Cerebral Aneurysm Formation

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Project and Objectives

Abstract

A cerebral aneurysm is a dilation of an artery in the brain, whose rupture can result in a hemorrhagic stroke. Studies have shown that degrading the extracellular matrix (ECM) protein, elastin, in part of an artery *in vivo* will induce an aneurysm (Cawley, 1996). However, the natural mechanism for aneurysm formation is unknown. Cerebral aneurysms occur in the arterial network called the Circle of Willis, which has complicated geometry, including complicated cell geometry. It is notable that cerebral aneurysms most often form at the apex of an artery bifurcation (Canham and Finlay, 2004), where smooth muscle cells are shorter and lack the spindle-like geometry found in nonbranching artery segments. To study cerebral aneurysm formation, we aimed to create confluent arterial tissue mimics with controlled cell organization and geometries corresponding to branching and nonbranching artery tissue. We used microcontact printing to generate tissue mimics consistent with different cell elongations by seeding smooth muscle cells onto "brick wall" ECM patterns. The elongation depended on the brick aspect ratio and cell density, thus we can vary these factors to create tissue mimics for further study.

Background

Studies have demonstrated that vascular smooth muscle cell (VSMC) shape affects cell phenotype expression and function (Alford et al., 2011), so our hypothesis is that cell shape also affects the expression of matrix metalloproteinases (MMPs), enzymes that degrade ECM proteins. From our perspective, the irregular geometry and mechanics of cells at the bifurcation site may induce MMP secretion, leading to degradation of elastin in the surrounding matrix, and initiating aneurysm formation.

In previous work we had tentatively established that when seeding human umbilical artery vascular smooth muscle cells (HUASMCs) on "brick wall" ECM patterns with varying aspect ratios, nuclear eccentricity increased with brick aspect ratio. However, when the tissues were seeded at high density, this relationship disappeared. We wanted to know if we could still generate tissue mimics with a low enough density to create differences in nuclear eccentricity but a high enough density for the cells to be confluent like they are *in vivo.* To do this, we examined how cell density affected nuclear eccentricity.

Methods

We used microcontact printing to construct 2-D arterial tissue mimics, as described previously (Alford et al., 2011; Sevcik, 2013). Briefly, a microfabricated silicon wafer is used to create a polydimethylsiloxane (PDMS) stamp resembling the wafer's negative space. The stamp is "inked" with the ECM protein fibronectin (50 ug/mL) solution, and incubated for one hour. The excess fibronectin solution is removed, leaving a thin layer, and the stamp is applied to a UVO treated PDMS substrate on a glass coverslip so the ECM pattern transfers. Cells are seeded onto the substrate and their integrin proteins form focal adhesions with the ECM, causing the cells to take on the geometry of the ECM pattern. In this study, HUASMCs were seeded onto ECM stamped in a "brick wall pattern". The cells were imaged after being fixed and stained with DAPI (chromatin) and phalloidin (f-actin). Custom MATLAB code was used to perform analysis of the actin alignment and nuclear shape and orientation.

In this project we were interested in the nuclear eccentricity because nuclear eccentricity is used to quantify cell geometry and elongation. Populations of cells with higher nuclear eccentricities are likely more elongated than populations of cells with lower nuclear eccentricities (Alford et al, 2011). This means that tissues containing shorter cells that have

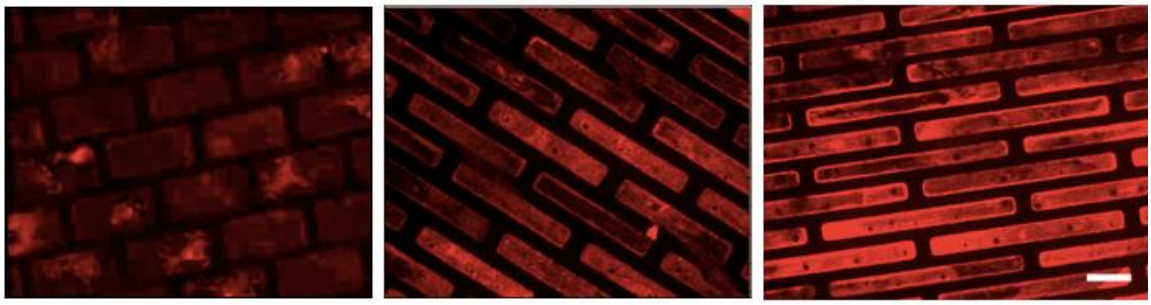

Figure 1: "Brick wall" ECM patterns with brick area 5000um^2 and brick aspect ratios of two, five, and ten (left to right). Scale bar is 50 um. Red: Fibronectin labeled with fluorescent antibody.

lower eccentricities can serve as mimics for arterial tissue at a bifurcation, whereas tissues containing longer cells that have higher eccentricities can serve as mimics for non-bifurcation tissue. We also examined the orientational order parameter (OOP) of the nuclei and f-actin filaments. A high OOP indicates the tissue is well-aligned, while a low OOP indicates more random alignment.

Results and Discussion

Our tissues had high nuclear and f-actin OOPs, indicating that the tissues were generally well

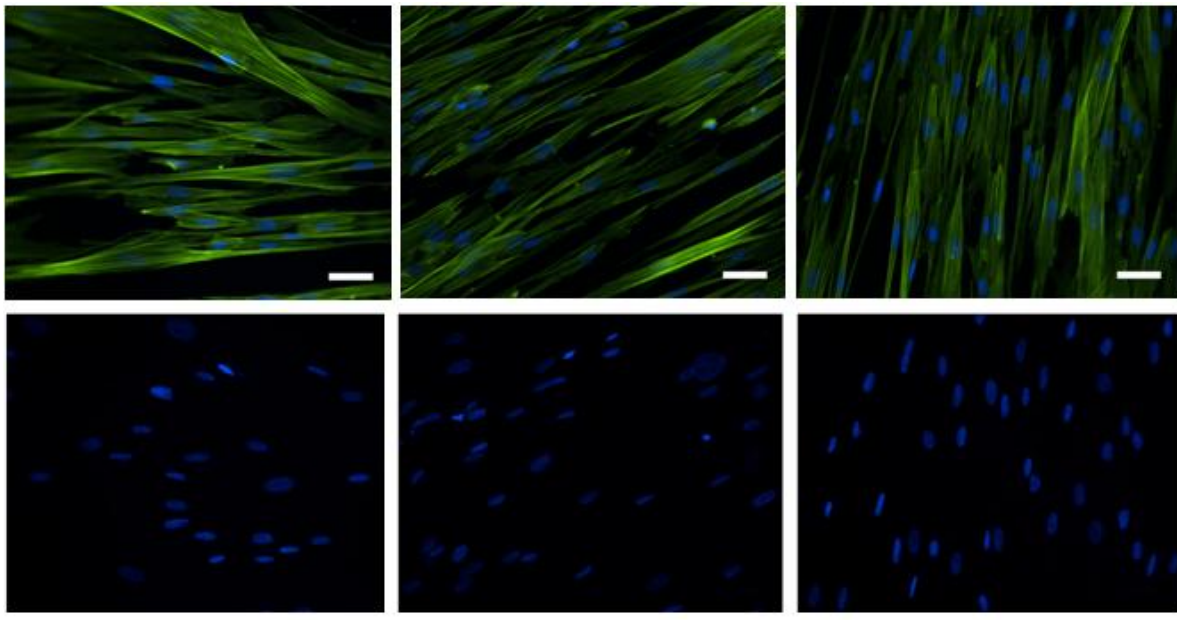

Figure 2: Representative images of cells seeded with 60,000 cells/well. Top: Tissues. Bottom: Nuclei. From left to right, tissues were seeded on ECM patterns with brick aspect ratio of two, five, and ten. Brick area was 5000 um^2, Scale bar is 50 µm. Green: phalloidin (f-actin). Blue: DAPI (chromatin).

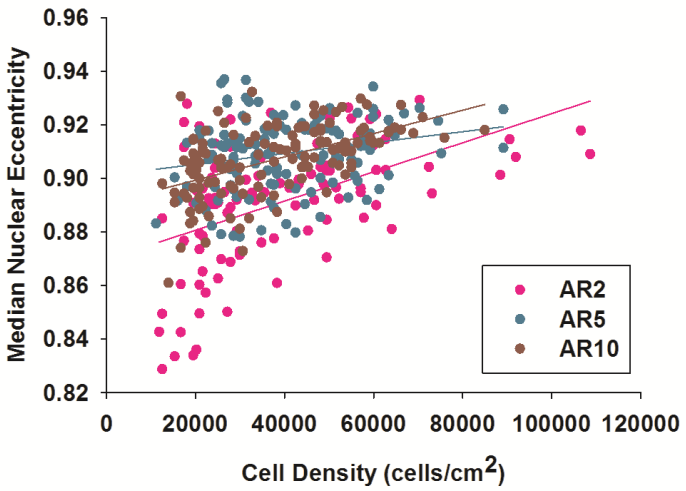

Figure 3: Nuclear eccentricity plotted against local cell density. A Pearson correlation was used to determine that the median eccentricity tends to increase with the local density for each aspect ratio ($p<0.05$). For AR2, $p=2.5 \times 10^{-7}$; for AR5, $p=0.0086$; and for AR10 $p=7.5 \times 10^{-11}$.

aligned, so nuclear eccentricity was the main difference between various tissues. Data were taken from several tissues with different seeding densities for brick wall ECM patterns with brick areas of 5000 um^2 and brick aspect ratios of two, five, and ten (Fig. 1). Representative images of tissues seeded on brick wall patterns are shown in Fig. 2, indicating we were able to use the brick wall ECM patterns to produce confluent tissues. A plot of local nuclear eccentricity versus local cell density is shown in Fig. 3. This shows how for a given brick aspect ratio, nuclear eccentricity increases with local cell density.

We aimed to determine how overall seeding density affected eccentricity, so in Fig. 4 data are shown for ten representative images of each tissue, as opposed to locally for single images. Seeding density is expressed as cells per $1.67cm^2$ well. For 50,000, 60,000, and 75,000 cells this well area corresponds to a density of 29,866, 35,839, and 44,799 cells/cm^2 respectively, although not all seeded cells may adhere. The cell density (Fig. 4 top) and nuclear eccentricity (Fig. 4 bottom) are shown for each tissue grouped by seeding density and by aspect ratio. Statistical significance is marked among tissues seeded at the same densities.

These results are consistent with the results shown in Fig.4. For 50,000 cells/well seeding density, densities are constant around 20,000 cells/cm^2 and nuclear eccentricity increases

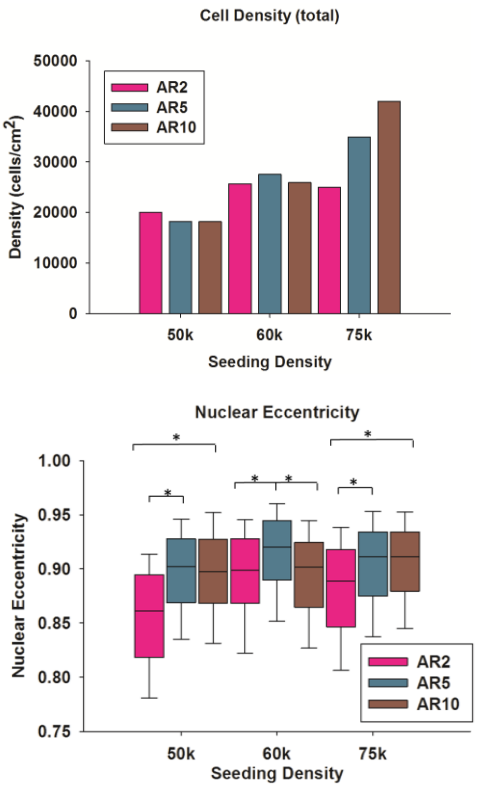

Figure 4: Top: Cell density versus seeding density. Bottom: nuclear eccentricity versus seeding density. * For nuclear eccentricity, a Kruskal-Wallis one way ANOVA on ranks showed statistically significant differences with $p < 0.001$

from aspect ratio two to aspect ratio five and then aspect ratio five and ten have similar values. This same nuclear eccentricity result is observed for 75,000 cells/well even though cell density was higher and increased coincidentally with aspect ratio, consistent with lower eccentricities for lower densities seeded on aspect ratio two patterns and similar higher eccentricities for aspect ratios five and ten. For 60,000 cells/well seeding density, the nuclear eccentricity for aspect ratio five is higher than the others, as expected from the data in Fig. 4. Notably, it is difficult to seed at a prescribed density because of random error and there can also be local variation in cell density within the tissue. This project accomplished the objectives of characterizing the relationship between cell density and nuclear eccentricity within arterial tissue mimics.

Reflection on UROP experience

Each semester I spend in the lab I learn more about many aspects of research and science. This semester I was able to learn more about different ways cells self-assemble on ECM patterns and how this knowledge can be applied to various tissue engineering applications. This semester I was also able to improve my seeding technique in the lab. I am fortunate that I was able to learn and do research through the Undergraduate Research Opportunities program.

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